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Contents

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THE USE OF RADIOPHOSPHORUS, P^{32}, TO MEASURE PHOSPHORUS UTILIZATION BY LAYING HENS[1]

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Abstract

Phosphorus utilization in four laying hens has been studied by means of radiophosphorus (P^{32}), which was incorporated into the laying mash as tricalcium phosphate replacing the bone meal in the diet. The results obtained indicate that, for these four hens:

1. The phosphorus that was absorbed appeared in the yolk, white, and shell within 24 hr. after feeding.

2. The maximum recovery of P^{32} following a single feeding was within 24 hr. in the case of the shell, 48 to 72 hr. for the white, and 144 hr. (six days) for the yolk.

3. A large portion of the unabsorbed phosphorus was excreted within 24 hr. of feeding.

4. A considerable quantity of the phosphorus absorbed by the digestive system was found to be stored in the tibiae at least 40 days after feeding.

5. The percentage uptake of phosphorus from tricalcium phosphate rose gradually in the egg and became relatively constant in about 14 to 15 days after the first feeding of tricalcium phosphate.

Introduction

It is well known that radiophosphorus, P^{32}, is a valuable tracer tool in studying phosphorus metabolism. A number of studies have already been made with hens. Hevesy and Hahn (7) injected radioactive disodium hydrogen phosphate into laying hens, which were killed some hours later. P^{32} was determined in various organs and in the egg and it was concluded that the bulk of the phosphatides in the yolk originated in the liver.

Entenman *et al.* (6) injected P^{32} as disodium hydrogen phosphate and killed the birds 12 hr. later. Eighteen yolks from four birds all contained a small amount of radiophosphorus.

Chargaff (3) injected radioactive sodium phosphate into laying hens and examined the phosphorus compounds in the yolks of the eggs for eight days

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following. The activity in the yolk increased to a maximum at the fifth day and then fell off.

Lorenz *et al.* (9) made a detailed study of the fate of P^{32} administered subcutaneously as sodium hydrogen phosphate. They found up to 0.59% of the P^{32} in the shell six hours after injection. The P^{32} reached a maximum of 2.5% in the yolk after about 130 hr. and a maximum of 0.2% in the albumen after about 60 hr.

Scott and others (5, 11) fed P^{32} as phosphoric acid and found about 23% excreted in a 60 day period. P^{32} was deposited mainly in the musculature and the bones with a shift from the former to the latter with time.

While the above experiments yield a great deal of information the method of administering phosphorus does not correspond very closely to that ordinarily practised in poultry feeding. Bone meal is a common source of phosphorus in poultry rations. Consequently it was thought that useful information might be obtained by replacing the bone meal in the mash with calcium phosphate containing P^{32}.

The P^{32} emits beta particles, which can be counted using a Geiger Counter, the rate of counting giving a measure of the P^{32} present and therefore of the original radioactive phosphate and the inactive phosphate with which it was originally mixed. Thus from the measured activity of say, the yolk, the percentage of phosphorus, in the yolk, that came from the added calcium phosphate in the feed, can be calculated. Naturally, due allowance must be made for self absorption of electrons by the material itself and for the decay of the P^{32} (half life 14.3 days) (8, pp. 82-94).

Experimental

Outline of Trials

Three birds from the University flock, all in laying condition, were fed a laying mash that had the following composition:

Ground wheat	20.0 lb.
Ground oats	25.0
Ground barley	20.0
Wheat bran	8.0
Wheat shorts	10.0
Meat meal (55%)	7.0
Whey powder	4.0
Vitagras	1.6
Bone meal	1.0
Limestone powder	3.0
Salt	0.5
Fish oil (1200 A/gm., 200 D/gm.)	1.0
Manganese sulphate	¼ lb./ton of mash

Throughout the trials, the bone meal was replaced with calcium phosphate containing P^{32}. To prevent loss of radioactivity through spillage, the mash was fed in a moistened condition. Each bird received 2 oz. of this wet mash and 2 oz. of whole grain (wheat and oats) daily. They also had access to a soluble calcium-bearing grit.

The first bird, a Barred Plymouth Rock hen, aged about 14 months, was isolated and fed daily doses of 700 mgm. of radioactive tricalcium phosphate in the mash, for four successive days (June 29 to July 2). All eggs laid after June 29 were hard boiled, separated into the yolk, white, and shell, wet ashed, and the radioactivity and total phosphorus determined. This bird was killed on Aug. 14. The left tibia was removed, cleaned of all adhering tissue, and wet ashed for the quantitative determination of P^{32}.

Both the second and third birds were New Hampshire pullets, aged about five months. The second bird received a single feeding of 700 mgm. of active calcium phosphate mixed in the laying mash on Aug. 17. All eggs laid after this date were hard boiled, separated into the yolk, white, and shell, wet ashed, and the radioactivity and total phosphorus determined. In addition, 24 hr. samples of droppings from the bird were collected in an enamel tray. The droppings were wet ashed and the total phosphorus and radioactivity determined.

The third bird was fed a daily dose of 700 mgm. of calcium phosphate for a period of 25 days (Aug. 12 to Sept. 5). The weight of active calcium phosphate in each 700 mgm. sample was adjusted so that the bird received the same amount of radioactivity every day, more active calcium phosphate being used as time went on to allow for the decay of P^{32} (see Fig. 1). The following is a record of the 25 calcium phosphate feedings:

Date P^{32} fed	Wt. of active $Ca_3(PO_4)_2$, mgm.	Wt. of inactive $Ca_3(PO_4)_2$, mgm.	Total weight, mgm.
Aug. 12	100	600	700
13	105	595	700
14	110	590	700
15	115	585	700
16	121	579	700
17	127	573	700
18	133	567	700
19	140	560	700
20	146	554	700
21	154	546	700
22	161	539	700
23	168	532	700
24	177	523	700
25	185	515	700
26	195	505	700
27	205	495	700
28	215	485	700
29	225	475	700
30	235	465	700
31	248	452	700
Sept. 1	260	440	700
2	274	426	700
3	287	413	700
4	303	397	700
5	317	383	700

Every second egg laid in this 25-day period was broken into a 400 cc. beaker and wet ashed for the determination of radioactivity and total phosphorus.

Preparation of Radioactive Calcium Phosphate

P^{32}, in the form of disodium hydrogen phosphate, was added to a suitable amount of disodium hydrogen phosphate solution. This was added to a slightly acid solution containing the calculated quantity of calcium nitrate.

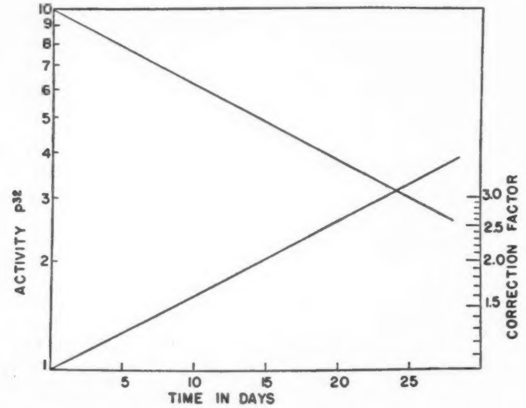

FIG. 1. *Upper curve, activity of P^{32} plotted on semilog paper (left ordinate) against time in days; lower curve, correction factor (right ordinate) to allow for decay of P^{32}. (Example: correction factor after one-half life, 14.3 days, is 2.)*

The solution was heated and calcium phosphate precipitated by the addition of ammonium hydroxide. The precipitate was washed and dried. The total phosphorus was determined and corresponded to that expected for calcium phosphate. The activity was determined using a Geiger-Müller Counter.

Method of Counting

A platinum dish containing the material to be counted was placed in a machined brass plate and slid under the window of a Geiger-Müller beta chamber having a thin mica window (3 mgm. per sq. cm.) at one end. Care was taken to ensure standard geometry. The chamber was connected to a scale of 128 scaling circuit and any given sample was counted for a sufficient length of time to give approximately 10,000 counts (probable error is then approximately 1%) (13). The background count was subtracted from the observed count. Changes in counter efficiency were allowed for by 'sandwiching' the unknown between counts of a uranium oxide standard. In some experiments, e.g. with bird No. 1, the decay of P^{32} was allowed for mathematically using the half life of 14.3 days (Fig. 1).

Activity of Calcium Phosphate Containing P^{32}

Self absorption by the calcium phosphate was allowed for by determining the activity of various weights of active calcium phosphate, spread uniformly in a small platinum dish (2.2 cm. diameter) (see Table I). Plotting the specific activity against the weight allows one to determine the specific activity for

TABLE I

ACTIVITIES OF VARIOUS WEIGHTS OF CALCIUM PHOSPHATE—JULY 29

Counts per minute	Weight, gm.	Counts per minute per mgm.	Correction factor
1560	0.0448	34.8	1.05
2042	0.0613	34.0	1.07
3060	0.0922	33.2	1.10
Extrapolated value for zero weight		36.5	1.00

zero sample thickness (Fig. 2). All counts were corrected to a uranium standard of 3200 counts per minute. The correction curve (Fig. 2) obtained from the last column of the following table agrees very closely with that for magnesium pyrophosphate (13).

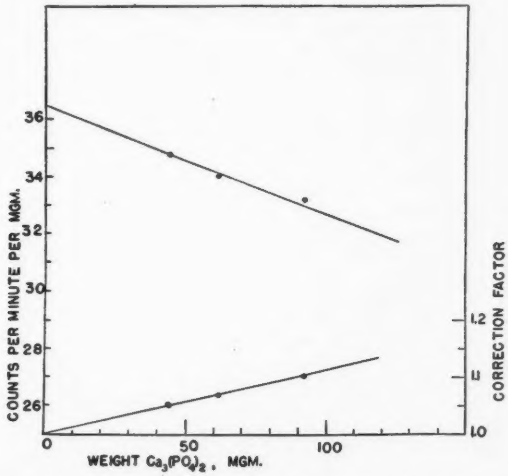

FIG. 2. *Upper curve, activity of sample of calcium phosphate in counts per minute per mgm. (left ordinate) plotted against weight of sample in mgm.; lower curve, correction factor to convert found activity to activity for sample of negligible thickness.*

For the samples from bird No. 2 (which received only a single feeding of P^{32}), the decay in the radioactivity was allowed for by 'sandwiching' each sample with a phosphorus standard containing a known weight of the original calcium phosphate. In the repeated dose experiment, the radioactive decay was already allowed for in the daily feeding of calcium phosphate, and therefore a phosphorus standard was not required. In this experiment counter variations were taken care of by sandwiching each sample with a uranium oxide standard. The results were calculated as the percentage uptake of the daily activity fed to the bird. A correction factor was applied to allow for

the decay in the period between the laying of the egg and the determination of its activity. Corrections for self absorption by the sample were applied (13, 8).

Activity of Repeated Dose Experiment

On Aug. 27, 17.4 mgm. of active calcium phosphate had an activity of 4860 counts per minute when the uranium oxide standard was 3108 counts per minute. Hence the activity fed on this date (205 mgm.), corrected to a uranium oxide standard of 3200 counts per minute,

$$= \frac{4860 \times 1.01 \times 3200 \times 205}{17.4 \times 3108} = 59{,}500 \text{ counts per minute.}$$

Similarly, on Sept. 4, 19.2 mgm. of calcium phosphate had an activity of 3620 counts per minute and the uranium oxide standard had 3040 counts per minute. The activity fed on this date (303 mgm.), corrected to a uranium oxide standard of 3200 counts per minute,

$$= \frac{3620 \times 1.01 \times 3200 \times 303}{19.2 \times 3040} = 61{,}000 \text{ counts per minute.}$$

The average $\left(\frac{59{,}500 + 61{,}000}{2}\right) = 6.02 \times 10^4$ counts per minute, is therefore the amount of activity fed to the bird on each of the 25 days. (With a counter efficiency of 25%, the total activity fed may be calculated to be approximately 3μc. (microcuries).)

Wet Ashing

The sample (yolk, white, shell, or the whole egg) was digested in 30 to 50 cc. of concentrated nitric acid on a hot plate for two to three hours. This brought the sample into solution except for a thin layer of fatty material observed in the yolk or the whole egg. Ten to fifteen cc. of concentrated sulphuric acid were then added to the yolk and the white, while about 10 cc. of perchloric acid were added to the shell and the whole egg. On continued heating, most of the nitric acid was boiled off and the solution turned black. This could be cleared by the addition of a small amount of concentrated nitric acid. By repeating the process of clearing the blackened solution with nitric acid, eventually all coloring matter is destroyed and a water white solution is obtained. To hasten the clearing process for the yolks and the whites, about 10 cc. of perchloric acid may be added in addition to the 10 to 15 cc. of concentrated sulphuric acid.

Droppings were dried and treated in the same manner as whole eggs.

Total Phosphorus

Total phosphorus was determined colorimetrically, on an aliquot of the wet ash solution, by the hydrazine sulphate method (12).

Determination of Radioactivity

The colorless solution from the wet ashing was diluted to 100 cc. and filtered. An aliquot was taken out, made basic to *p*-dinitrophenol with ammonium

hydroxide and then an equal volume of 2.5*N* nitric acid was introduced. On addition of 10 cc. of ammonium molybdate solution* the yellow ammonium phosphomolybdate precipitated. This precipitate was digested at about 60° C. for 30 min. before it was filtered, dissolved in 6*N* ammonium hydroxide solution and precipitated by 10 cc. of magnesia mixture** plus 5 to 10 cc. of concentrated ammonium hydroxide solution. After digestion at room temperature overnight the magnesium ammonium phosphate was collected by centrifuging and transferred to a platinum dish on which it was dried and ignited to magnesium pyrophosphate and weighed before the sample was counted under the Geiger-Müller counter.

Self-Absorption by the Precipitate

Self-absorption by the magnesium pyrophosphate precipitate was allowed for as in (13). Actually, the correction factor is the same as for calcium phosphate as in Fig. 2.

Results

Bird No. 1

Typical calculation: egg No. 4, laid July 4.

Yolk wet ashed and solution made up to 100 cc. Phosphate from 50 cc. aliquot converted to magnesium pyrophosphate (162 mgm.) and counted July 16 at 1 p.m., 328 counts per minute. Correction factor for self-absorption = 1.17, uranium standard 3310 counts per minute.

Total counts per minute based on a uranium standard of 3200 counts per minute is $\frac{328 \times 1.17 \times 3200 \times 2}{3310} = 744$ counts per minute.

Four feeds of calcium phosphate, each 0.7 gm., activity = 81 counts per minute per mgm. as of 9 a.m. July 11. Total = 2.27×10^5 counts per minute. Correction factor to allow for decay by July 16, 1 p.m. given by

$$\log \frac{n_0}{n} = \frac{0.693t}{2.3 \times 14.3} = \frac{0.693 \times 5.17}{2.3 \times 14.3} = 0.1089$$

$$\text{and } n = \frac{n_0}{1.288} = \frac{2.27 \times 10^5}{1.288} = 1.76 \times 10^5$$

$$\text{and recovery of phosphorus in yolk} = \frac{744 \times 100}{1.76 \times 10^5} = 0.42\% .$$

The percentage recovery of P^{32} from the shell, white, and yolk of eggs laid by bird No. 1 is given in Table II and Fig. 3.

The percentage recovery of P^{32} from the left tibia of this hen 42 days after feeding was 6.7%.

* *The ammonium molybdate solution was prepared by dissolving 90 gm. of ammonium molybdate in 100 cc. of ammonium hydroxide solution, adding 240 gm. of ammonium nitrate and diluting to a total volume of 1 liter.*

** *The magnesia mixture was prepared by dissolving 50 gm. of magnesium chloride and 100 gm. of ammonium chloride in 500 cc. of distilled water made slightly ammoniacal with ammonium hydroxide. This was allowed to stand overnight, filtered, made slightly acid, and diluted to 1 liter.*

TABLE II

RECOVERY OF P^{32} LAID BY BIRD No. 1

Egg No.	Egg laid		Recovery of total P^{32} fed, %			Total P, mgm. (colorimetric)		
	Date	Time	Shell	White	Yolk	Shell	White	Yolk
1	June 30	11:30 a.m.	0.064	0.006	0.001	6.8	3.8	Lost
2	July 1	Noon	0.055	0.018	0.054	7.0	3.5	106.0
3	July 2	4:30 p.m.	0.125	0.025	0.180	9.3	3.8	106.0
4	July 4	11:30 a.m.	0.051	0.032	0.420	8.3	4.1	116.2
5	July 5	3:00 p.m.	0.026	0.036	0.510	6.8	4.5	120.0
6	July 6	3:45 p.m.	0.010	0.008	Lost	8.3	3.8	107.3
7	July 8	11:00 a.m.	*	*	0.398			124.5
8	July 9	6:30 p.m.			0.356			121.5
9	July 10	2:00 p.m.			0.234			114.8
10	July 11	4:30 p.m.			0.161			121.5
11	July 13	11:00 a.m.			0.130			123.0
12	July 14	11:00 a.m.			0.127			131.3
14	July 17	12 noon			0.080			121.5
16	July 20	3:30 p.m.			0.040			117.0
18	July 23	3:00 p.m.			0.040			117.0

* *No measurable activity.*

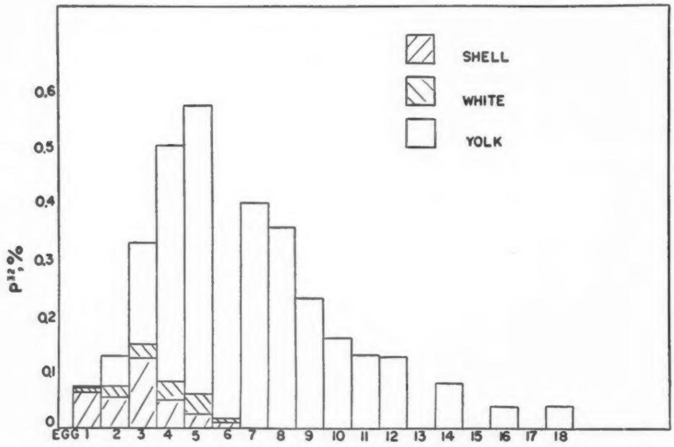

Fig. 3. *Histogram showing recovery of P^{32} in shell, white, and yolk of successive eggs, expressed as a percentage of the total P^{32} fed. P^{32} fed as active calcium phosphate on four successive days.*

Bird No. 2

Typical calculation: egg No. 3, yolk. Phosphorus converted to magnesium pyrophosphate, activity 820 counts per minute.

Sample weight = 77.6 mgm. Therefore correction factor (Fig. 2) = 1.08.

Standard phosphate sample weight 19.2 mgm., activity 3604 counts per minute; correction factor = 1.01.

$$\text{Uptake of } P^{32} = \frac{820 \times 1.08}{3604 \times 1.01} \times \frac{19.2}{700} \times 100 = 0.66\% .$$

The following is the weight and date of eggs laid by bird No. 2:

Egg No.	Date of laying	Weight of egg, gm.
1	Aug. 19	53
2	21	50
3	22	50
4	24	50
5	26	50
6	28	51
7	30	54
8	Sept. 1	53
9	3	54
10	5	57
11	6	54

The recovery of activity from the various parts of the eggs laid by bird No. 2 is given in Table III and Fig. 4.

TABLE III

EGGS FROM BIRD NO. 2, PERCENTAGE UPTAKE OF PHOSPHORUS FROM CALCIUM PHOSPHATE

Egg number and part	Uptake of P^{32}, %	Total P, mgm.
Yolk No. 1	0.05	72.0
2	0.25	73.0
3	0.66	77.0
4	0.95	73.0
5	—*	—*
6	0.64	94.8
7	0.35	87.2
8	0.19	88.0
9	0.12	88.0
10	0.13	96.0
11	0.12	90.0
White No. 1	0.08	3.2
2	0.16	3.4
3	0.07	4.3
4	0.06	4.0
5	0.02	4.0
6	0.22	5.0
7	0.04	5.0
Shell No. 1	0.36	5.2
2	0.13	7.6
3	0.05	6.2
4	0.89**	7.0
5	0.07	6.9
6	0.02	7.5
8	0.02	6.8

* *Lost on ashing.*

** *Probably contaminated during ashing.*

Whites, Nos. 8 to 11, and shells, Nos. 7, 9 to 11, had no measurable activity.

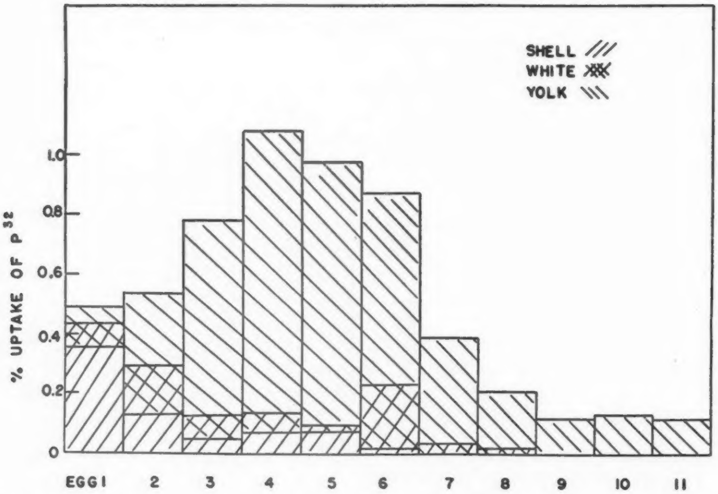

FIG. 4. *Histogram showing recovery of P^{32} in shell, white, and yolk of successive eggs, expressed as a percentage of the total P^{32} fed. P^{32} fed as a single feeding of active calcium phosphate.*

The total phosphorus and the percentage of P^{32} excreted in each days' droppings for bird No. 2 are shown in Table IV.

TABLE IV

PERCENTAGE PHOSPHORUS FROM CALCIUM PHOSPHATE APPEARING IN DROPPINGS—BIRD No. 2

Date of droppings	Excretion of P^{32}, %	Total P, mgm.
Aug. 18	15.90	552
19	2.39	420
20	1.92	450
21	1.03	480
22	0.63	400
23	0.44	528
24	0.21	397
25	0.20	429
26	0.07	461
27	0.50	528
28	0.18	395
29	0.06	502
30	0.00	415

Since it was possible that there might have been some contamination of the droppings of Aug. 18 and 19, the experiment was repeated with bird No. 4. These results are recorded in Table V.

TABLE V

PERCENTAGE PHOSPHORUS FROM CALCIUM PHOSPHATE APPEARING IN DROPPINGS—BIRD NO. 4

Date of droppings	Excretion of P^{32}, %	Date of droppings	Excretion of P^{32}, %
Sept. 26	8.66	Sept. 28	0.58
Sept. 27	0.74	Sept. 29	0.00

Bird No. 3

Typical calculation: egg No. 9, laid Aug. 23.

Counted Sept. 4, total activity 1098 counts per minute.*

Sample weight = 68.4 mgm. Correction factor for self-absorption by sample is 1.06.

Decay factor, 12 days, is 1.77.

Daily activity fed = 60,200 counts per minute (see page 168)*.

$$\% \text{ recovery} = \frac{1098 \times 1.06 \times 1.77}{60{,}200} \times 100 = 3.42 \text{ .}$$

The percentage uptake of phosphorus for bird No. 3 is shown in Table VI and Fig. 5.

TABLE VI

EGGS FROM BIRD NO. 3, PERCENTAGE UPTAKE OF PHOSPHORUS (REPEATED DOSE EXPERIMENT)

Egg number	Date of laying	Weight of egg, gm.	Uptake of P^{32}, %	Total P, mgm.
1	Aug. 12	40	0.25	71.5
2	15	45	0.86	68.0
4	17	42	2.66	72.5
5	19	46	3.16	77.5
7	21	50	3.98	84.5
9	23	50	3.42	100.0
11	25	46	4.34	90.0
13	27	48	3.78	104.8
15	30	52	3.83	104.0
17	Sept. 1	45	3.76	104.0
19	4	47	3.55	96.0

Discussion

In these experiments, P^{32} was fed either in repeated doses or as a single dose in order to determine the fate of phosphorus when fed to laying birds. By referring to Table III, it will be seen that phosphorus is deposited in the egg within 24 hr. of feeding. A large proportion of this phosphorus is located in the shell. It is interesting to note that the percentage recovery in the shell decreases sharply after the first feeding. The amount of P^{32} recovered from

* *Corrected to a uranium oxide standard of 3200 counts per minute.*

the yolk and white within the first 24 hr. after feeding is relatively small since these components of the egg are almost completely formed some 20 hr. before laying (14). As each succeeding egg is laid, the total amount of P^{32} recovered

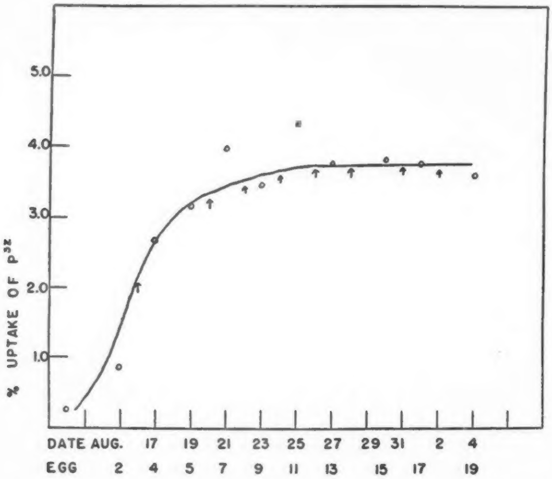

FIG. 5. *Recovery of P^{32} in successive whole eggs expressed as a percentage of the P^{32} fed on any one day. Same activity of P^{32} fed as active calcium phosphate for 25 successive days. (Eggs not analyzed indicated by an arrow.)*

from the yolk increases at a fairly uniform rate. This is in agreement with the results of Lorenz *et al.* (9) and Chargaff (3). The fact that the peak of recovery is approximately six days after feeding would indicate that this is the length of time for the yolk to pass through the late stage of greatly accelerated growth. This substantiates the findings of Riddle (10). With respect to the white, the maximum recovery was noted within 24 to 72 hr., which is in accord with Lorenz *et al.* (9).

The analyses of the eggs from bird No. 1 (Table II) follow the same general trend but are not so distinct since she was fed four successive doses of P^{32}.

However, the marked break in the curve occurring at egg No. 10 in Fig. 3 is interesting. It is known that the yolk is laid down in about 10 days and thus an egg laid more than 10 days after the activity was administered could not have obtained its activity directly from the feed but only indirectly, for example, from the tissue and skeletal matter. This is in agreement with the general idea of 'exchange' of phosphorus compounds within the body and finds support in the fact that the left tibia of bird No. 1 showed 6.7% of the P^{32} fed, 42 days after feeding.

The experiment in which the bird is fed a single dose of labelled tricalcium phosphate shows very clearly that this single dose is eventually distributed through a larger number of eggs.

The phosphorus in any given egg must, of course, come from the phosphorus fed on many different days, and from a material balance, we can say that, for regular feeding and laying, the total phosphorus from the feed appearing in any one egg should equal the total utilization of any one day's feed. The situation is illustrated graphically in Fig. 6. The fate of the phosphorus fed on successive days is shown by the full curves, the total phosphorus obtained on successive days being given by the sum of these curves i.e. by the broken line curve in Fig. 6. It evidently agrees very well with experiment (Fig. 5).

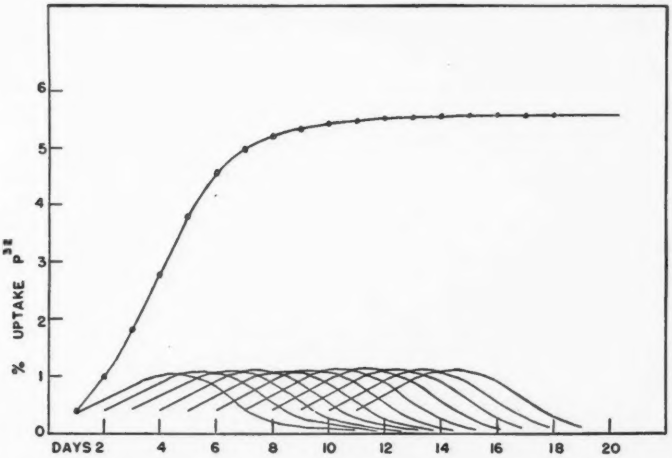

FIG. 6. *Theoretical curve showing recovery of P^{32} (as percentage of daily P^{32} fed) in successive eggs in multiple feeding experiment as a summation of recoveries in successive single feeding experiments.*

The percentage uptake of phosphorus from a specific source (in this case active calcium phosphate) rises gradually in the egg (Table VI and Fig. 5) and becomes relatively constant in 14 or 15 days after the first feeding. Cook *et al.* (5) fed radioactive phosphoric acid to growing birds and studied the percentage uptake of P^{32} in different organs at varying lengths of time after feeding. However, no work seems to have been reported on the time required for tagged phosphorus to reach a constant level in the eggs of birds that are laying at a uniform rate of production.

The ashing of the tibia of bird No. 1 indicated that there is a large amount of phosphorus stored in the bones. Further studies on laying birds similar to that reported by Cook *et al.* (5) with growing birds should prove worth-while.

The results obtained from the analysis of the droppings of bird No. 2 indicate that the biggest excretion of phosphorus is within 24 hr. after feeding (see Table IV). The amount of excretion of the P^{32} (and this is indicative of the phosphorus excreted that was fed as calcium phosphate) decreases very rapidly after the first 24 hr. following feeding, and is negligible in about 12

days. The results of ashing the droppings of bird No. 4 (Table V) would indicate an even faster rate of decrease in excretion of phosphorus following the first 24 hr. after feeding. This may be an individual characteristic of each hen. Lorenz *et al.* (9) found a marked irregularity in the excretion of P^{32}. Our results are in agreement with those of Common (4) and Lorenz *et al.* (9).

Since the birds were fed ground limestone in the mash and had access to a soluble calcium-bearing grit, the amount of tricalcium phosphate had no apparent effect on egg shell quality as was noted by Buckner *et al.* (1, 2) when they fed precipitated tricalcium phosphate as the only mineral supplement. These authors concluded that poor egg shells were produced when tricalcium phosphate was fed as the only mineral supplement because calcium was a limiting factor. This would not be the case in these experiments since both ground limestone and a soluble calcium-bearing grit were fed.

Further investigations with radioactive minerals are in progress at the present time.

Acknowledgments

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TOXICITY OF SELECTED ORGANIC COMPOUNDS TO INSECTS

PART I. TESTS FOR GENERAL TOXICITY ON LARVAE OF *MUSCA*, *TRIBOLIUM*, AND *EPHESTIA*, AND ADULTS OF *SITOPHILUS*[1]

By A. W. A. Brown,[2] D. B. W. Robinson,[2] H. Hurtig,[2] and B. J. Wenner[2]

Abstract

The general toxicity of 127 synthetic organic compounds was tested against larvae of *Musca domestica*, *Tribolium confusum*, and *Ephestia kuehniella*, and adults of *Sitophilus granarius*. The compounds were mixed in the insects' food in graded concentrations, and their toxicity was assessed by determination of the median lethal concentrations (LC_{50}) for each of the four species. The most highly toxic compounds were gammexane (the gamma isomer of hexachlorocyclohexane) and chlordane (obtained by distillation of technical chlordane). The toxicity of DDT was on the average one-half of that of the first two compounds, and it was superior to any of the 12 analogues tested. Four chlorinated aliphatic hydrocarbons, namely hexachloropropene, hexachlorobutadiene, and the *symm*-and *asymm*-heptachloropropanes, showed a high toxicity related to their powerful fumigant action. A high level of toxicity was shown by benzyl thiocyanate and its chlorinated derivatives. The nitro compounds dinitro-*o*-cresol, nitrostyrene, dinitrodimethylbutane and dinitrocyclohexylphenol were especially toxic to *Sitophilus* adults, but were ineffective against *Musca* larvae. Certain aromatic semicarbazones recommended by previous workers gave disappointing results. Of 22 derivatives of morpholine tested, only three showed any degree of toxicity to the four species of insects employed.

Introduction

A program of synthesis of selected organic compounds for tests on certain species of insects has been undertaken at the Experimental Station, Suffield, Alta. The 127 compounds that were selected for investigation fell mainly in the following groups: chlorinated aliphatic and cyclic hydrocarbons, cyanides, and nitriles, thiocyanates, derivatives of morpholine, and compounds analogous to DDT. Certain compounds outside these classifications were also included.

This paper covers the initial screening tests for general toxicity of the compounds to four species of insects. In this type of test the compound is intimately mixed with the food medium in which the insect lives and feeds. It thus provides an over-all measure of the contact, stomach, and fumigant toxicity of the compound, without distinction as to the mode of action. The compounds were later tested for contact toxicity to three species of insects (see Part II* of this series). Testing of their stomach toxicity alone was not undertaken.

It was considered that these compounds, which were carefully selected to contain molecular structures that might prove insecticidal, were worthy of a

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thorough type of investigation. Accordingly the insects on which they were tested were representative of several orders, namely the Orthoptera, Hemiptera, Lepidoptera, Diptera, and Coleoptera, and examples of larvae and nymphs as well as adults were employed. In this way the possibility of passing over an insecticide by reason of using an unsuitable test insect species or developmental stage was minimized. Moreover it was felt that the data might yield information on the species or group specificity of toxic action of a particular compound or class of compounds.

Throughout these investigations the compounds were tested at a number of concentrations, which were progressively reduced until the resulting mortality was less than 20%. From the percentage mortality figures the median lethal concentration (here treated synonymously with the term LC_{50} as the point at which 50% mortality is expected) and the LC_{90} were calculated for each compound.

Material

The biological material consisted of larvae of the housefly (*Musca domestica* L.), the confused flour beetle (*Tribolium confusum* Duval), and the Mediterranean flour moth (*Ephestia kuehniella* Zeller), and adults of the granary weevil (*Sitophilus granarius* L.). They were taken from stocks continuously reared in the laboratory at 78° F. and 65% relative humidity. The stock of *Musca* was reared according to the standard Peet-Grady technique (8). *Tribolium* was reared on whole wheat flour in open enamel trays, and *Ephestia* was reared on corn meal and whole wheat flour in gauze-topped battery jars. The cultures of *Sitophilus* were kept continuously on hard wheat grains in battery jars.

The larvae of *Musca* employed in the tests were obtained by inoculating newly-laid eggs on the standard rearing medium and incubating them for two days at 78° F. In the case of *Tribolium* and *Ephestia* the adults were confined over sieved household flour, and the larvae were obtained 10 to 14 days later by sieving the flour, when they were 7 to 11 days old. The adults of *Sitophilus* were taken from the culture two to four weeks after emergence.

The organic compounds to be tested had been synthesized or specially purified for the purpose. The majority (93) were produced by Dr. D. B. W. Robinson and Mr. J. B. Reesor of the Experimental Station, Suffield, and are designated by the prefix ESS. Eighteen compounds were submitted by Prof. G. F Wright, Department of Chemistry, University of Toronto, and bear the prefix UT. Eight compounds were obtained from the Department of Chemistry, McGill University, and are denoted by McG. An additional eight compounds were kindly supplied by Mr. C. P. Clausen, Bureau of Entomology and Plant Quarantine, U.S. Department of Agriculture, and are designated by the prefix USDA.

The sample of chlordane (1) was obtained by double distillation of technical chlordane (the Technical 1068 of the Velsicol Corporation). The sample of

isoborneyl thiocyanoacetate was obtained by distillation of the insecticide Thanite of the Hercules Powder Company.

The sample of dinitro-*o*-cresol employed (ESS 62) showed a melting point of 88° C. This compound is termed 4,6-dinitro in the Handbook of Chemistry and Physics and the Dictionary of Organic Compounds (Heilbron & Bunbury), but is usually referred to as 3,5-dinitro in commercial specifications and in current entomological literature.

Methods

The test media were prepared by intimately mixing the compounds with the food in proportions ranging from 0.64% (of the air-dry weight of the food material) down to the lowest dilution of 0.000005% (i.e. 6400 down to 0.05 parts per million). Each successive dilution was one-half of the preceding one, and tests were performed with it if the mortality at the preceding concentration exceeded 20%. In all cases 4 oz. specimen jars with screw tops of copper screening were employed. The compounds were finely ground in a mortar, added to the medium, and mixed thoroughly by revolving the containers on eccentric rollers. For concentrations below 0.2%, the compound was added in acetone solution and the solvent was evaporated during mixing under a blast of hot air from a portable hair-drier.

For *Musca* larvae, the test medium consisted of 23 gm. of bran and alfalfa in the ratio of 2 to 1, to which 30 cc. of an aqueous solution containing 6.7% of malt extract (Bynin) and 0.05% of dried brewer's yeast (Dow N.B.) was added and mixed to form a homogeneously moist mass. Each sample thus prepared was inoculated with 50 young larvae. Two or three replicates were set up for every concentration of each compound. The test samples were placed in an incubator at 80° F., and after three days they were examined and the fresh pupae and live larvae remaining were counted. Control mortality in this medium was found to be 0.2% on the basis of 36 control samples set up parallel with the tests.

For larvae of *Ephestia* or *Tribolium*, the test medium consisted of 20 gm. of whole wheat flour. Each test sample was prepared in duplicate and was inoculated with 20 young larvae. They were held at 80° F. for five to six weeks, and were then examined for mortality. The survivors were in the larval or pupal stages. Parallel control samples were run with each group of tests; the average control mortality for *Tribolium* was 3.7% out of 59 control samples, and for *Ephestia* it was 4.1% out of 49 control samples. One group of tests with *Tribolium* and two with *Ephestia* were discarded owing to high control mortality.

The procedure in which adults of *Sitophilus* were used was based on the method reported by Swingle, Phillips, and Gahan (13). The test medium consisted of 15 gm. of hard wheat grains coated with a known amount of the compound under test. Each sample was inoculated with 25 *Sitophilus*

adults, and the mortality was recorded after a period of four days at 80° F. Tests were performed for the most part in duplicate. The average control mortality, out of 70 control samples, was 3.5%.

Results

The percentage mortalities obtained with successive dilutions of the 127 compounds tested against the four species of insects will not be reported fully. However, the results obtained with the 15 compounds that proved to be most toxic for *Musca* are shown in detail in Table I, to indicate the type of data obtained. No correction was applied to compensate for the low control mortality rate.

TABLE I

MORTALITY DATA FOR THE 15 MOST TOXIC COMPOUNDS TESTED AGAINST *Musca* LARVAE

Average control mortality: 0.2%

Group	Name of compound	Percentage mortality at the following concentrations in p.p.m.									
		6400	3200	1600	800	400	200	100	50	25	12.5
B	Chlordane	100	100	100	94	100	100	100	100	60	0
A	*s*-Heptachloropropane	100	100	100	95	95	89	96	65	62	6
B	Gammexane	100	100	100	100	100	100	87	77	12	—
B	Benzotrichloride	100	100	100	94	90	96	88	2	1	—
A	Hexachloropropene	100	100	100	100	100	76	20	3	—	—
B	*p*-Chlorobenzyl chloride	100	100	98	91	86	80	4	—	—	—
B	*o*-Chlorobenzyl chloride	100	100	100	100	62	39	11	9	—	—
L	Benzyl thiocyanate	100	100	100	96	78	27	14	4	—	—
A	*as*-Heptachloropropane	100	100	100	93	90	39	5	—	—	—
D	*o*-Chlorobenzyl cyanide	100	100	99	99	45	33	19	—	—	—
D	Phenylacetonitrile	100	100	96	91	88	13	12	—	—	—
D	Phthalonitrile	100	100	100	89	66	17	10	—	—	—
D	Benzonitrile	100	100	100	88	17	21	4	—	—	—
L	*o*-Chlorobenzyl thiocyanate	100	98	100	74	50	22	19	—	—	—
D	2,4-Dichlorobenzyl cyanide	99	98	99	83	20	12	—	—	—	—

The approximate LD_{50} and LD_{90} for each compound was obtained by plotting the logarithm of the percentage mortality by the method of Bliss (2). In practice the concentration and mortality figures were plotted directly on logarithmic–probit graph paper (Winthrop Chemical Co.). The percentage concentrations corresponding to 50 and 90% mortalities could be read directly from the regression lines thus obtained.

The values for the LC_{50} and LC_{90} derived for each of the 127 compounds for the four species employed are tabulated in Table II. The figures are expressed in parts per million, an LC_{50} of 6400, for example, indicating 50% mortality at a concentration of 0.64%. Where the LC_{90} or LC_{50} was in excess of 7000 p.p.m. the symbol "Neg" is used to indicate nontoxicity at that level. The symbol "—" indicates that the compound, due to its scarcity, was not tested against the species concerned.

TABLE II

APPROXIMATE MEDIAN LETHAL CONCENTRATION (LC_{50}) AND LC_{90} FIGURES OF 127 COMPOUNDS FOR LARVAE OF *Musca, Tribolium,* AND *Ephestia,* AND ADULTS OF *Sitophilus*

Expressed in parts per million, by weight

No.	Name of compound	*Musca*		*Sitophilus*		*Tribolium*		*Ephestia*	
		LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Chlorinated aliphatics									
ESS 107	1,1,1-Trichloroethane	Neg	Neg	Neg	Neg	Neg	Neg	5700	Neg
ESS 139	2,3-Dichloropropane	Neg	Neg	3000	Neg	Neg	Neg	5700	Neg
ESS 134	2,3-Dichloropropene-1	2900	5000	Neg	Neg	Neg	Neg	5000	Neg
ESS 83	1,2,3-Trichloropropane	1600	2300	Neg	Neg	3500	5000	1800	3600
ESS 56	Hexachloropropene	140	250	450	950	10	110	4	7
ESS 37	*asymm*-Heptachloropropane	250	580	360	500	30	350	80	230
ESS 100	*symm*-Heptachloropropane	38	100	450	870	400	1300	55	220
ESS 81	Octachloropropane	900	1800	1290	2300	Neg	Neg	200	450
ESS 118	1,4-Dichlorobutane	3300	5200	Neg	Neg	3200	4600	2100	3400
ESS 57	Hexachlorobutadiene-1,3	2800	Neg	2200	3800	15	200	25	300
Halogenated cyclic compounds									
McG 2	*p*-Dichlorobenzene	2800	4400	Neg	Neg	3200	6000	800	2400
ESS 90	Gamma-hexachlorocyclohexane	44	90	0.1	0.25	3	18	10	35
McG 1	*p*-Chlorobenzaldehyde	—	—	1450	2400	Neg	Neg	Neg	Neg
ESS 111	*o*-Chlorobenzyl chloride	220	480	1100	1840	280	4000	1800	3500
ESS 112	*p*-Chlorobenzyl chloride	160	440	3500	Neg	350	2200	1800	2800
ESS 132	2,4-Dichlorobenzyl chloride	620	1500	1250	1900	2200	3500	2300	4000
ESS 133	3,4-Dichlorobenzyl chloride	1000	1850	1300	2200	2600	4200	2100	3900
ESS 109	Benzotrichloride	77	130	1160	1830	500	900	800	1900
ESS 108	Benzotrifluoride	6500	Neg	1850	3050	Neg	Neg	6400	Neg
ESS 74	*p*-Chloroacetophenone	1400	2900	580	1000	1650	2700	2600	4500
ESS 75	*p*-Chloropropiophenone	3200	4600	560	1000	3500	5200	5200	Neg
ESS 110	α-Chloro-1-methylnaphthalene	2300	4200	210	380	4000	5100	1900	3500
UT 6	5-Chlorofurfural	5300	Neg	1500	2700	Neg	Neg	Neg	Neg
UT 7	5-Bromofurfural	7000	Neg	1650	2600	Neg	Neg	Neg	Neg
UT 1	Cholesteryl chloride	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
UT 8	Chlordane	23	34	1.3	4.5	0.2	1.3	36	75
DDT and analogues									
ESS 33	*p,p'*-Dichlorodiphenyltrichloroethane	700	1600	16	41	16	210	860	2700
McG 3	*p,p'*-Diiododiphenyltrichloroethane	Neg	Neg	800	Neg	Neg	Neg	Neg	Neg
ESS 69	*p,p'*-Dimethyldiphenyltrichloroethane	Neg	Neg	1200	2100	Neg	Neg	1600	Neg
McG 6	*p,p'*-Dibromomethyldiphenyltrichloroethane	7000	Neg	1900	Neg	Neg	Neg	Neg	Neg
ESS 72	*p,p'*-Dimethoxydiphenyltrichloroethane	Neg	Neg	840	1700	Neg	Neg	500	1800
ESS 71	*p,p'*-Diethoxydiphenyltrichloroethane	1900	3900	1900	2600	110	5000	450	1800
ESS 70	*p,p'*-Dichlorodiphenyldichloroethylene	Neg	Neg	1550	3300	2700	6500	1300	3500
McG 7	Carbinol DDT benzoate	—	—	—	—	—	—	Neg	Neg

TABLE II—*Continued*

APPROXIMATE MEDIAN LETHAL CONCENTRATION (LC_{50}) AND LC_{90} FIGURES OF 127 COMPOUNDS FOR LARVAE OF *Musca, Tribolium,* AND *Ephestia,* AND ADULTS OF *Sitophilus*—Continued

Expressed in parts per million, by weight

No.	Name of compound	*Musca*		*Sitophilus*		*Tribolium*		*Ephestia*	
		LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
DDT and analogues—Concluded:									
ESS 19	Trichloroethylidene-bis-benzamide	Neg	Neg	2300	4200	Neg	Neg	Neg	Neg
ESS 18	Trichloroethylidene-bis-phenacetamide	Neg	Neg	2900	5600	Neg	Neg	Neg	Neg
UT 12	*p,p'*-Dichlorobenzophenone oxime	Neg	Neg	1300	Neg	Neg	Neg	Neg	Neg
McG 8	bis(Dichlorothienyl)-trichloroethane	—	—	1350	2200	Neg	Neg	Neg	Neg
ESS 55	4,4'-bis(N-Benzylanilino)-methane	Neg	Neg	Neg	Neg	Neg	Neg	—	—
Cyanides and nitriles									
ESS 85	Octanoyl nitrile	2100	2700	Neg	Neg	5400	Neg	3100	5000
ESS 86	Decanoyl nitrile	4200	7000	4230	Neg	3800	6500	3700	5000
ESS 87	Tetradecanoyl nitrile	4000	8000	970	1850	Neg	Neg	6000	Neg
ESS 88	Octadecanoyl nitrile	Neg	Neg	1950	3600	Neg	Neg	3300	5000
ESS 1	Benzonitrile	400	750	1700	2500	6500	Neg	800	3800
ESS 7	Benzonitrile hexachloride	Neg	Neg	—	—	—	—	—	—
USDA 2	Phthalonitrile	320	760	360	910	900	4000	770	3500
ESS 2	Phenylacetonitrile	310	850	660	1040	4500	Neg	2000	Neg
ESS 24	*p*-Nitrophenylacetonitrile	2800	Neg	800	1220	5200	Neg	1100	3800
ESS 103	*o*-Chlorobenzyl cyanide	270	650	580	1000	3400	4800	2600	4700
ESS 104	*p*-Chlorobenzyl cyanide	640	1250	200	500	300	2500	3100	4500
ESS 135	2,4-Dichlorobenzyl cyanide	550	960	1100	2000	250	6000	5100	Neg
ESS 137	3,4-Dichlorobenzyl cyanide	750	1100	590	1050	2000	3200	4000	5500
ESS 9	β-Phenylpropionitrile	780	1850	940	1350	6000	Neg	Neg	Neg
UT 4	Styryl cyanide	1300	2100	107	250	1100	2100	2400	4000
ESS 101	α-Cyano-1-methylnaphthalene	3100	5100	520	970	Neg	Neg	5500	Neg
N-Methyl carbamates:									
ESS 116	of *m*-diethylaminophenol methochloride	Neg	Neg	1600	3500	Neg	Neg	Neg	Neg
ESS 115	of *m*-diethylaminophenol methiodide	Neg	Neg	1500	2900	Neg	Neg	Neg	Neg
ESS 117	of *m*-diethylaminophenol methosulphate	Neg	Neg	1750	3050	Neg	Neg	Neg	Neg
BSS 114	of 2-methyl-5-dimethylamino-phenol methochloride	Neg	Neg	1330	2550	Neg	Neg	Neg	Neg
ESS 113	of 2-methyl-5-dimethylamino-phenol methiodide	Neg	Neg	1450	2800	2500	4000	1400	3400
Semicarbazones:									
USDA 3	of ethyl methyl ketone	—	—	1010	1720	Neg	Neg	3000	4500
USDA 4	of 2-furaldehyde	—	—	1800	3800	Neg	Neg	3300	5000
USDA 5	of cyclopentanone	2700	6600	1300	2100	Neg	Neg	3800	5000

TABLE II—*Continued*

APPROXIMATE MEDIAN LETHAL CONCENTRATION (LC_{50}) AND LC_{90} FIGURES OF 127 COMPOUNDS FOR LARVAE OF *Musca, Tribolium,* AND *Ephestia,* AND ADULTS OF *Sitophilus*—Continued

Expressed in parts per million, by weight

No.	Name of compound	*Musca*		*Sitophilus*		*Tribolium*		*Ephestia*	
		LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Semicarbazones:—Concluded									
USDA 6	of 2,4-dimethyl-3-pentanone	—	—	1250	2000	Neg	Neg	Neg	Neg
USDA 7	of cyclohexanone	—	—	1350	2100	Neg	Neg	3000	4500
USDA 8	of *p*-chloroacetophenone	—	—	1350	2000	Neg	Neg	3200	4500
Morpholine compounds									
ESS 10	Morpholine	Neg	Neg	980	1850	5200	Neg	Neg	Neg
ESS 12	N-Ethylmorpholine	Neg	Neg	4150	Neg	Neg	Neg	Neg	Neg
ESS 14	N-Butylmorpholine	5300	Neg	2500	4000	Neg	Neg	230	1350
ESS 73	N-Trichoroacetylmorpholine	Neg	Neg	820	2500	550	5500	Neg	Neg
ESS 15	N-Phenylmorpholine	Neg	Neg	600	1500	3100	Neg	1150	4000
ESS 89	N-(*p*-Chlorophenyl)morpholine	6000	Neg	1000	1280	Neg	Neg	2300	3900
ESS 17	N-Benzoyl morpholide	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
ESS 29	N-(*p*-Tolyl)morpholine	Neg	Neg	150	310	3400	Neg	1060	3000
ESS 30	N-(*m*-Tolyl)morpholine	Neg	Neg	260	700	4800	Neg	800	2500
ESS 43	N-(*p*-Chlorobenzoyl) morpholide	Neg	Neg	2000	3500	Neg	Neg	Neg	Neg
ESS 45	N-(*o*-chlorobenzoyl) morpholide	Neg	Neg	990	Neg	Neg	Neg	Neg	Neg
ESS 65	N-(*m*-Nitrobenzoyl) morpholide	Neg	Neg	1900	Neg	Neg	Neg	Neg	Neg
ESS 26	Benzenesulphonyl morpholide	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
ESS 27	*p*-Bromobenzenesulphonyl morpholide	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
ESS 28	*p*-Toluenesulphonyl morpholide	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
ESS 49	Morpholine-N-sulphonyl anilide	Neg	Neg	1300	2800	Neg	Neg	Neg	Neg
ESS 50	Morpholine-N-sulphonyl *p*-chloroanilide	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
ESS 52	Morpholine-N-sulphonyl 2,4-dichloroanilide	Neg	Neg	6000	Neg	Neg	Neg	Neg	Neg
ESS 53	Morpholine-N-sulphonyl α-naphthylamide	Neg	Neg	2400	Neg	Neg	Neg	Neg	Neg
ESS 54	Morpholine-N-sulphonyl β-naphthylamide	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
ESS 51	N,N′-Dimorpholino sulphone	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
ESS 67	Morpholine picrate	Neg	Neg	920	1800	Neg	Neg	1900	Neg
Nitro compounds									
UT 10	2,3-Dinitro-2,3-dimethyl-butane	Neg	Neg	84	160	1000	2200	2600	4500
ESS 62	3,5-Dinitro-*o*-cresol	Neg	Neg	18	35	950	1750	130	400
ESS 46	β-Nitrostyrene	630	1550	67	170	1100	2000	1100	3600
ESS 20	*o*-Nitrobiphenyl	Neg	Neg	220	640	Neg	Neg	1750	Neg
ESS 21	*p*-Nitrobiphenyl	Neg	Neg	1060	Neg	Neg	Neg	Neg	Neg
UT 13	2,4-Dinitro-6-cyclohexyl-phenol	2500	Neg	110	195	4700	Neg	1750	4800

TABLE II—*Concluded*

APPROXIMATE MEDIAN LETHAL CONCENTRATION (LC_{50}) AND LC_{90} FIGURES OF 127 COMPOUNDS FOR LARVAE OF *Musca, Tribolium,* AND *Ephestia,* AND ADULTS OF *Sitophilus*—Concluded

Expressed in parts per million, by weight

No.	Name of compound	*Musca*		*Sitophilus*		*Tribolium*		*Ephestia*	
		LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Miscellaneous nitrogenous compounds									
ESS 25	*symm*-Diphenylguanidine	Neg	Neg	6500	Neg	Neg	Neg	Neg	Neg
UT 9	Dibutylnitramine	2000	3600	1010	2060	5200	Neg	2700	4200
UT 5	Dicyanodiethylnitramine	Neg	Neg	1400	3000	Neg	Neg	Neg	Neg
UT 11	Di-*n*-butylcyanamide	1550	3200	290	720	3300	Neg	1800	3200
ESS 22	*o*-Aminobiphenyl	Neg	Neg	750	1200	2700	4400	1100	3600
UT 2	Jablonski compound $C_4H_6N_4O$	Neg	Neg	7000	Neg	Neg	Neg	Neg	Neg
UT 3	Jablonski compound $C_4H_8N_4O_2$	Neg	Neg	7000	Neg	Neg	Neg	Neg	Neg
Compounds containing C, H, and O only									
ESS 58	Cyclohexylacetic acid	Neg	Neg	600	1000	Neg	Neg	Neg	Neg
ESS 59	Cyclohexylpropionic acid	Neg	Neg	270	450	Neg	Neg	Neg	Neg
ESS 60	Cyclohexylbutyric acid	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
ESS 61	Cyclohexylcaproic acid	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
ESS 23	*o*-Hydroxybiphenyl	7000	Neg	860	1320	Neg	Neg	6000	Neg
ESS 3	Coumarin	1400	2900	75	200	4000	5400	1350	4500
Thiocyanates									
ESS 41	Benzyl thiocyanate	230	580	68	140	55	500	330	770
ESS 105	*o*-Chlorobenzyl thiocyanate	400	830	60	135	1600	3400	320	970
ESS 106	*p*-Chlorobenzyl thiocyanate	700	1550	94	205	1800	3600	550	2000
ESS 136	2,4-Dichlorobenzyl thiocyanate	1600	3300	640	5000	570	1000	400	1600
ESS 138	3,4-Dichlorobenzyl thiocyanate	Neg	Neg	520	1600	2200	3400	900	2900
ESS 102	α-Thiocyano-1-methyl-naphthalene	Neg	Neg	1210	2000	Neg	Neg	1500	3500
UT 14	Isoborneyl thiocyanoacetate	1700	3500	220	750	—	—	—	—
Miscellaneous sulphur compounds									
ESS 82	β,β'-Dichlorodiethyl sulphoxide	850	2600	2450	Neg	5500	Neg	3400	4800
UT 16	bis(*p*-Chlorophenyl)sulphone	6000	Neg	Neg	Neg	—	—	—	—
McG 5	bis(*p*-Bromophenyl)sulphone	—	—	2100	4500	—	—	Neg	Neg
McG 4	*p*-Bromophenyl sulphonic acid	6300	Neg	Neg	Neg	Neg	Neg	Neg	Neg
ESS 91	Thiourea	3900	Neg	Neg	Neg	4800	7000	400	1300
USDA 1	Thiocoumarin	2300	4300	1050	1310	Neg	Neg	1200	2100
Compounds containing other elements									
UT 17	Phenylboric acid	700	1450	102	305	50	450	150	270
UT 18	*o*-Nitrophenylboric acid	—	—	1700	Neg	—	—	—	—
ESS 68	Tri-*o*-cresyl phosphate	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
ESS 63	Pyridyl mercuric stearate	3600	Neg	710	1100	Neg	Neg	1500	Neg

Discussion of Results

The results obtained with chlordane and with gammexane are very similar, both compounds showing median lethal concentrations of less than 50 parts per million to all four species. The median lethal concentration of 44 p.p.m. for gammexane against *Musca* larvae by this method is high in comparison with the value of 8 p.p.m. found for benzene hexachloride by McGovran and Piquett (10), who assessed the mortality at the emergence of the adult.

DDT showed a generally high rating, being superior to its analogues. The low toxicity found for methyl-DDT is surprising in view of the excellent results obtained with it against *Pediculus* and *Cimex* by Busvine (4). However, Proverbs and Morrison (11) found that methyl-DDT showed extremely low residual contact toxicity to *Drosophila*. It is also remarkable that methoxy-DDT, recommended for household use because it is alleged to be as toxic as DDT to insects but much less toxic to mammals, showed no toxicity whatever to larvae of *Musca* and *Tribolium*.

The four chlorinated aliphatic hydrocarbons: hexachloropropene, hexachlorobutadiene, and the heptachloropropanes, show a high toxicity by these methods of testing. Although they exhibit no contact toxicity (see Part II of this series of papers) they possess fumigant activity of a high order (unpublished data). Thus their activity is most marked in the case of *Musca*, *Tribolium*, and *Ephestia*, whose larvae live within the closely-packed, nonaerated test medium, whereas in the better ventilated tests with *Sitophilus* adults, these compounds are relatively ineffective.

In addition to phenylboric acid, benzyl thiocyanate would appear to be the most promising new compound revealed by these general tests. It was slightly more toxic than its chloro derivatives and considerably more toxic than its dichloro analogues. Of the five chlorinated analogues of methylbenzene tested, benzotrichloride is most effective, followed by the *o*-chloro and the *p*-chlorobenzyl chlorides.

Of the six nitro compounds tested, four of them were quite strongly insecticidal, while the two nitrobiphenyls were virtually nontoxic. *Sitophilus* in the adult stage was the most susceptible species to these nitro compounds, which include DNOC (dinitro-*o*-cresol), nitrostyrene, dinitrodimethylbutane, and DNOCHP (dinitrocyclohexylphenol). On the other hand, *Musca* larvae showed the least reaction to them, no nitro compounds being included in the 15 most toxic compounds for this species (see Table I), and both DNOC and dinitrodimethylbutane being entirely without effect. The high toxicity of dinitrodimethylbutane is of interest in view of its relationship with 2-nitrobutane, which has shown marked fumigant effect to *Tribolium confusum* (12). The general high rating of β-nitrostyrene is of interest in view of the good results recently obtained by American workers with halogenated derivatives of the closely related nitroethylbenzenes (13).

Of the 15 most toxic compounds to *Musca* larvae, five are cyanides or nitriles. This class of compounds is not represented in the 15 most toxic

compounds for either *Sitophilus* or *Ephestia*. Benzonitrile is in general exceeded in toxicity by phenylacetonitrile (i.e. benzyl cyanide). Of the chlorinated derivatives of benzyl cyanide, the *o*-chloro is more effective against *Musca* and *Ephestia*, while the *p*-chloro is the more toxic against *Tribolium* and *Sitophilus*. The 2,4-dichlorobenzyl cyanide is slightly more effective on the average than the 3,4- derivative. In tests against *Ephestia* and *Tribolium* the chlorinated benzyl cyanides and chlorides were liable to give inconsistent results.

The *p*-nitro derivative of benzyl cyanide (*p*-nitrophenylacetonitrile) was of a lower order of toxicity than the *p*-chloro analogue in these experiments, although it has shown a high order of toxicity to larvae of *Cochliomyia* (3), *Prodenia* (13), and two species of *Culex* (5 and 7). Of the other cyanides tested, phthalonitrile showed a consistently good level of toxicity to all four species, the median lethal concentration obtained for *Musca* being in close agreement with that obtained by McGovran and Piquett (9) by similar methods. Styryl cyanide and the aliphatic nitriles showed moderate to light toxicity.

Thiocoumarin gave results that were consistently inferior to those obtained with coumarin itself. It may be noted that coumarin showed a marked repellent action to *Musca* larvae. The semicarbazones of aromatic ketones, which gave promise in the experiments performed by Swingle, Gahan, and Phillips (14), did not show any outstanding toxicity in these tests, which were made as far as the quantity of available material allowed. As a group the five N-methyl carbamates tested gave poor results. Neither of the two so-called Jablonski compounds, which are cyanamide-formaldehyde condensation products of uncertain composition and doubtful stability, showed any significant toxicity. Of the biphenyls tested, the *o*-amino derivative was found to give the best results; this compound was found to give 50% mortality of *Anopheles* larvae at less than 1 p.p.m. (6). Thiourea was found to be moderately toxic to *Ephestia*, but weakly toxic to the other species, including *Musca*, where the results were inferior to those obtained by McGovran and Piquett (9).

The only morpholine derivatives to show any considerable degree of general toxicity to all four species were the *p*- and *m*-tolylmorpholines. Butylmorpholine showed an unusually high toxicity to *Ephestia* larvae. Trichloroacetyl morpholide was markedly toxic to *Tribolium* larvae. Phenylmorpholine, trichloroacetyl morpholide and morpholine itself gave good results against *Sitophilus* adults.

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TOXICITY OF SELECTED ORGANIC COMPOUNDS TO INSECTS

PART II. TESTS FOR CONTACT TOXICITY ON NYMPHS OF *BLATELLA* AND *ONCOPELTIS*, AND ADULTS OF *TRIBOLIUM*[1]

BY A. W. A. BROWN,[2] B. J. WENNER,[2] AND F. E. PARK[2]

Abstract

The direct contact toxicity of 91 synthetic organic compounds was tested against nymphs of *Blatella germanica* and of *Oncopeltis fasciatus*, and adults of *Tribolium confusum*. The compounds were dissolved in graded concentrations in benzene–kerosene mixture and sprayed on the insects in a spraying tower. Their toxicity was assessed by determination of the median lethal deposits (LD_{50}) for each of the three species.

Taking the results with the three species as a whole, the highest contact toxicity was shown by gammexane and chlordane. Dinitro-*o*-cresol and dinitrocyclohexylphenol were next in order of effectiveness. These were followed by benzyl thiocyanate and its *o*- and *p*-chloro and 2,4-dichloro derivatives. DDT was sixth and methoxy-DDT was 12th on the list of compounds in order of their average effectiveness to the three species. A number of chlorinated aliphatic compounds that were strong fumigants showed no contact toxicity.

Introduction

Of the 127 organic compounds tested for general toxicity to insects and reported in Part I of this series, 91 were sufficiently soluble and in good enough supply to be tested for direct contact toxicity. They were dissolved in a mixture of benzene and kerosene and were applied to three species of insects in a spray tower.

Material

The biological material consisted of fourth and fifth instar nymphs of the German cockroach (*Blatella germanica* L.) reared continuously in the laboratory on compressed meat–vegetable pellets with yeast supplement; last instar nymphs of the large milkweed bug (*Oncopeltis fasciatus* Dall.) continuously reared in the laboratory on seeds of *Asclepias syriaca;* and adults of the confused flour beetle (*Tribolium confusum* Duval) reared continuously in the laboratory on whole wheat flour.

The nymphs of *Blatella* used in the tests were taken after development for 30 to 35 days after hatching at 80° F. and 70% relative humidity. The nymphs of *Oncopeltis* were taken 16 to 19 days after hatching. The adults of *Tribolium* were taken 15 to 25 days after emergence.

The organic compounds to be tested had been synthesized or specially purified for the purpose. The majority (77) were produced by Dr. D. B. W. Robinson and Mr. J. B. Reesor of the Experimental Station, Suffield, Alta., and are designated by the prefix ESS. Fourteen compounds were submitted by Prof. G. F Wright, Department of Chemistry, University of Toronto, and bear the prefix UT.

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Methods

The compounds to be tested were dissolved in a mixture of four parts by volume of benzene (reagent grade) and one part of odorless kerosene, the latter oil being added to reduce the volatility of the solvent. Spraying of the oil solutions was carried out in a cylindrical glass spraying tower measuring 29 in. high by 12 in. internal diameter*. The methods followed were in general similar to those described by Tattersfield and Morris (9) and by Busvine (3).

The spray solution was introduced from a 10 cc. burette into the aspirator attachment of a DeVilbiss Special Atomizer No. 631 centrally placed in the lid of the tower. Compressed air was applied to the atomizer at a pressure of 23 cm. Hg measured accurately by an open manometer. The stopcock of the burette was then opened, and the solution passed through a capillary constriction into the atomizer at the rate of 7 cc. per minute.

Four cc. of spray solution were delivered in each case. The room in which spraying was performed was kept at a temperature of 75° to 80° F. and a relative humidity of over 75%, in order to reduce the electrostatic charges generated in the tower. Under these conditions, 4 cc. of spray solution was found to yield an average deposit at the base of the cylinder of approximately 4 cu. mm. of solution per square centimeter. This density of deposit was determined by colorimetric assessment of dyed spray, and prevailed over the central 8 in. of the tower, decreasing toward the walls of the cylinder. Thus where 8% solutions were employed, the density of deposit of the dissolved compound would be 320 gamma per $cm.^2$ The average weight of the roaches employed was approximately 50 mgm., and their surface area exposed to the vertical fall of droplets was approximately 0.5 sq. cm. Thus for a deposit of 100 gamma per $cm.^2$, the contact dosage would be 50 gamma per roach, corresponding to 10 mgm. per kgm. body weight. The figures for surface dosage were obtained by printing the outline of the insect on sensitized paper and checked by recovery of dyed spray from a sprayed insect.

The tower was secured against air leaks by a wide band of adhesive tape applied at the junction of the friction lid with the top of the cylinder, and by closing the door by which the test insects were introduced. A half-inch opening in the lid holding a coarse esparto filter served to relieve excess air pressure in the tower. The spray was allowed to settle for one and one-half minute. After each spray test, the burette and atomizer were rinsed with acetone while an evacuating fan drew air towards the base of the tower; after a group of tests the tower was removed and the inner walls were cleaned with acetone.

The size of droplets in the spray was assessed by exposing microscope slides coated with the silicone preparation sold as G. E. Drifilm No. 9987. The diameter of the flattened droplets (lenses) thus obtained were measured under

** A convenient supply of glass cylinders is offered by obsolete gasoline pumps, and may be obtained from the Imperial Oil Company.*

the microscope by means of an ocular micrometer. The spreading coefficient, being the ratio between the lens diameter and the diameter of the spherical droplet, was determined according to the method described by May (7). By this method, the droplets were found to range between 5 and 90 μ in diameter, and the median diameter by mass was computed to be 62 μ.

The nymphs of *Blatella* and *Oncopeltis* were immobilized before spraying. After being exposed to a temperature of 34° F. to immobilize them in their rearing containers, they were transferred to Buchner funnels into which carbon dioxide gas was introduced for two minutes. This narcotic treatment kept the insects quiescent for the subsequent 10 to 15 min., during which time they were transferred to crystallizing dishes, placed in a normal posture, exposed in the spray tower, and returned to the observation jars. As may be seen from the control figures reported below, the narcotic treatment was without permanent effect on the insects; nymphs of both species that had been submitted to cold and then to carbon dioxide for 20 min. recovered completely.

The insects were exposed in crystallizing dishes measuring $2\frac{1}{2}$ in. high by $4\frac{3}{4}$ in. in diameter, fitted with a floor of filter paper or paper towelling; 20 nymphs of *Blatella* and of *Oncopeltis*, and 50 adults of *Tribolium* were employed in each test. After being sprayed, the insects were transferred to 2-qt. sealers fitted with screened tops, and were kept at a temperature of 80° F. and 70% relative humidity. The food supplied was meat–vegetable pellets and a vial of water for *Blatella*, milkweed seed and water for *Oncopeltis*, and a 2-in. layer of whole wheat flour for *Tribolium*. Mortality counts of *Blatella* were made 20 hr. later, the period being extended to three days in the case of DDT and its analogues, gammexane, and chlordane. The observation period for *Oncopeltis* was two days, being extended to four days for the above-mentioned slow insecticides. The observation period for *Tribolium* was three days.

The series of sprays performed with each compound was begun by applying 4 cc. of an 8% (wt./vol.) solution, and was continued with successive dilutions of 4, 2, 1, 0.5, 0.125, 0.062, 0.031, and 0.016%, as results warranted proceeding further. Thus the deposits obtained were successively halved with each dilution, whereas the amount of the solvent remained constant. The density of deposit would thus range from 320 gamma per $cm.^2$, through 160, 80, 40, etc. down to 0.062 gamma per $cm.^2$ Fifteen of the original 127 compounds, not mentioned in this paper, could not be tested owing to their insolubility in liquids suitable for spraying. Twenty-one compounds were not available in sufficient quantity. The boric acid derivatives (UT 17 and 18) were sprayed in a mixture of equal parts of kerosene and 1,4-dioxane. The N-methyl carbamates (ESS 113 to 117) were first dissolved in one part of methyl alcohol and diluted with three parts of the benzene–kerosene mixture.

Of 10 control samples of *Blatella* sprayed with 4 cc. of the solvent alone, the average mortality was 3.5%. Of eight similar control samples of *Oncopeltis*, the average mortality was 0.0%. Of five control samples of *Tribolium*, the mortality was zero.

Results

The percentage mortalities obtained with successive dilutions of the 91 compounds tested against the three species of insects will not be reported here. However, the results obtained with the 20 compounds that showed the greatest contact toxicity to *Blatella* nymphs are shown in Table I.

TABLE I

MORTALITY DATA FOR THE 20 COMPOUNDS THAT WERE MOST TOXIC BY DIRECT CONTACT TO *Blatella* NYMPHS

Average control mortality: 3.5%

Name of compound (often abbreviated)	% Mortality at the following deposits in gamma/cm.^{2}									
	320	160	80	40	20	10	5	2.5	1.25	0.62
Chlordane	100	100	100	100	100	95	82	75	30	20
Gammexane	100	100	100	100	100	100	70	48	20	—
Dinitro-*o*-cresol	100	100	100	100	88	85	62	10	—	—
Dinitrocyclohexylphenol	100	100	80	70	65	45	15	—	—	—
p-Chlorobenzyl thiocyanate	100	100	85	62	35	10	—	—	—	—
Benzyl thiocyanate	100	98	100	60	15	—	—	—	—	—
Benzyl cyanide	100	100	98	58	3	—	—	—	—	—
DDT	100	90	60	43	37	22	0	—	—	—
o-Chlorobenzyl thiocyanate	100	100	75	75	5	—	—	—	—	—
o-Chlorobenzyl cyanide	90	95	85	15	—	—	—	—	—	—
o-Hydroxybiphenyl	95	98	72	28	10	—	—	—	—	—
p-Chlorobenzyl cyanide	100	90	25	20	—	—	—	—	—	—
p-Chloropropiophenone	98	90	52	12	—	—	—	—	—	—
β-Nitrostyrene	100	70	40	25	16	—	—	—	—	—
Phenylpropionitrile	76	60	52	17	—	—	—	—	—	—
o-Aminobiphenyl	95	80	15	—	—	—	—	—	—	—
Styryl cyanide	100	90	5	—	—	—	—	—	—	—
3,4-Dichlorobenzyl cyanide	62	50	50	10	0	—	—	—	—	—
α-Chloromethylnaphthalene	100	75	15	—	—	—	—	—	—	—
Isoborneyl thiocyanoacetate	80	70	30	5	—	—	—	—	—	—

From the mortality figures so obtained, the median lethal deposit (LD_{50}) and the LD_{90} figures were derived for each compound. This was accomplished by plotting the logarithm of the deposit in gamma per cm.^{2} against the probit of the percentage mortality, according to the method of Bliss (1).

The values for LD_{50} and LD_{90} for each of the 91 compounds against the three species employed are tabulated in Table II. The figures are expressed in gamma (μgm.) of the compound per square centimeter of horizontal surface. Where the LD_{90} or LD_{50} was in excess of 350 gamma per cm.^{2} the symbol "Neg" is used to denote nontoxicity at that level. The symbol "–" indicates that the compound, owing to its scarcity or to its insolubility in suitable solvents, was not tested against the species concerned.

TABLE II

APPROXIMATE MEDIAN LETHAL DEPOSITS (LD_{50}) AND LD_{90} FIGURES OF 92 COMPOUNDS FOR NYMPHS OF *Blatella* AND *Oncopeltis*, AND ADULTS OF *Tribolium*

Expressed in gamma per sq. cm.

No.	Name of compound	*Blatella*		*Oncopeltis*		*Tribolium*	
		LD_{50}	LD_{90}	LD_{50}	LD_{90}	LD_{50}	LD_{90}
Chlorinated aliphatics							
ESS 107	1,1,1-Trichloroethane	Neg	Neg	—	—	Neg	Neg
ESS 139	2,3-Dichloropropane	Neg	Neg	Neg	Neg	Neg	Neg
ESS 134	2,3-Dichloropropene-1	Neg	Neg	Neg	Neg	Neg	Neg
ESS 83	1,2,3-Trichloropropane	Neg	Neg	Neg	Neg	Neg	Neg
ESS 56	Hexachloropropene	Neg	Neg	Neg	Neg	Neg	Neg
ESS 100	*symm*-Heptachloropropane	400	Neg	Neg	Neg	Neg	Neg
ESS 37	*asymm*-Heptachloropropane	340	Neg	Neg	Neg	Neg	Neg
ESS 81	Octachloropropane	Neg	Neg	Neg	Neg	Neg	Neg
ESS 118	1,4-Dichlorobutane	Neg	Neg	Neg	Neg	Neg	Neg
ESS 57	Hexachlorobutadiene-1,3	Neg	Neg	Neg	Neg	Neg	Neg
Halogenated cyclic compounds							
ESS 90	Gammexane	2.8	5.5	5.2	24	41	130
ESS 111	*o*-Chlorobenzyl chloride	Neg	Neg	Neg	Neg	Neg	Neg
ESS 112	*p*-Chlorobenzyl chloride	320	Neg	Neg	Neg	Neg	Neg
ESS 132	2,4-Dichlorobenzyl chloride	Neg	Neg	320	Neg	Neg	Neg
ESS 133	3,4-Dichlorobenzyl chloride	Neg	Neg	Neg	Neg	Neg	Neg
ESS 109	Benzotrichloride	320	Neg	Neg	Neg	Neg	Neg
ESS 108	Benzotrifluoride	Neg	Neg	Neg	Neg	Neg	Neg
ESS 74	*p*-Chloroacetophenone	210	270	Neg	Neg	Neg	Neg
ESS 75	*p*-Chloropropiophenone	80	185	Neg	Neg	Neg	Neg
ESS 110	α-Chloro-1-methylnaphthalene	120	205	Neg	Neg	Neg	Neg
UT 6	5-Chlorofurfural	Neg	Neg	Neg	Neg	Neg	Neg
UT 7	5-Bromofurfural	260	Neg	Neg	Neg	Neg	Neg
UT 1	Cholesteryl chloride	Neg	Neg	Neg	Neg	Neg	Neg
UT 8	Chlordane	1.7	6.5	70	150	50	240
DDT and analogues							
ESS 33	*p,p'*-Dichlorodiphenyltri-chloroethane	40	150	Neg	Neg	27	60
ESS 69	*p,p'*-Diiododiphenyltri-chloroethane	330	Neg	Neg	Neg	Neg	Neg
ESS 70	*p,p'*-Dichlorodiphenyldi-chloroethylene	Neg	Neg	Neg	Neg	Neg	Neg
ESS 72	*p,p'*-Dimethoxydiphenyltri-chloroethane	200	Neg	110	380	Neg	Neg
ESS 71	*p,p'*-Diethoxydiphenyltri-chloroethane	400	Neg	330	Neg	Neg	Neg
UT 16	bis(*p*-Chlorophenyl)sulphone	Neg	Neg	—	—	Neg	Neg
Cyanides and nitriles							
ESS 85	Octanoyl nitrile	Neg	Neg	Neg	Neg	Neg	Neg
ESS 86	Decanoyl nitrile	Neg	Neg	Neg	Neg	Neg	Neg
ESS 87	Tetradecanoyl nitrile	370	Neg	Neg	Neg	Neg	Neg
ESS 88	Octadecanoyl nitrile	340	Neg	Neg	Neg	Neg	Neg

TABLE II—*Continued*

APPROXIMATE MEDIAN LETHAL DEPOSITS (LD_{50}) AND LD_{90} FIGURES OF 92 COMPOUNDS FOR NYMPHS OF *Blatella* AND *Oncopeltis*, AND ADULTS OF *Tribolium*—Continued

Expressed in gamma per sq. cm.

No.	Name of compound	*Blatella*		*Oncopeltis*		*Tribolium*	
		LD_{50}	LD_{90}	LD_{50}	LD_{90}	LD_{50}	LD_{90}
Cyanides and nitriles							
ESS 1	Benzonitrile	Neg	Neg	Neg	Neg	Neg	Neg
ESS 2	Benzyl cyanide	35	95	Neg	Neg	Neg	Neg
ESS 103	*o*-Chlorobenzyl cyanide	55	115	Neg	Neg	Neg	Neg
ESS 104	*p*-Chlorobenzyl cyanide	80	160	320	Neg	Neg	Neg
ESS 135	2,4-Dichlorobenzyl cyanide	430	Neg	Neg	Neg	Neg	Neg
ESS 137	3,4-Dichlorobenzyl cyanide	100	Neg	Neg	Neg	Neg	Neg
ESS 9	β-Phenylpropionitrile	105	Neg	Neg	Neg	Neg	Neg
UT 4	Styryl cyanide	120	190	Neg	Neg	Neg	Neg
ESS 101	α-Cyano-1-methylnaphthalene	Neg	Neg	Neg	Neg	Neg	Neg
N-Methyl carbamates							
ESS 116	of *m*-Diethylaminophenol methochloride	120	Neg	—	—	Neg	Neg
ESS 115	of *m*-Diethylaminophenol methiodide	Neg	Neg	—	—	Neg	Neg
ESS 117	of *m*-Diethylaminophenol methosulphate	Neg	Neg	—	—	Neg	Neg
ESS 114	of 2-Methyl-5-dimethylamino-phenol methochloride	Neg	Neg	—	—	Neg	Neg
ESS 113	of 2-Methyl-5-dimethylamino-phenol methiodide	Neg	Neg	—	—	Neg	Neg
Morpholine compounds							
ESS 10	Morpholine	Neg	Neg	Neg	Neg	Neg	Neg
ESS 12	N-Ethylmorpholine	Neg	Neg	Neg	Neg	Neg	Neg
ESS 14	N-Butylmorpholine	Neg	Neg	Neg	Neg	Neg	Neg
ESS 73	N-(Trichloroacetyl) morpholide	380	Neg	Neg	Neg	Neg	Neg
ESS 15	N-Phenylmorpholine	Neg	Neg	Neg	Neg	Neg	Neg
ESS 89	N-(*p*-Chlorophenyl) morpholine	380	Neg	Neg	Neg	Neg	Neg
ESS 17	N-Benzoyl morpholide	Neg	Neg	Neg	Neg	Neg	Neg
ESS 29	N-(*p*-Tolyl)morpholine	165	Neg	Neg	Neg	Neg	Neg
ESS 43	N-(*p*-Chlorobenzoyl) morpholide	Neg	Neg	Neg	Neg	Neg	Neg
ESS 45	N-(*o*-Chlorobenzoyl) morpholide	Neg	Neg	Neg	Neg	Neg	Neg
ESS 26	Benzenesulphonyl morpholide	Neg	Neg	Neg	Neg	Neg	Neg
ESS 27	*p*-Bromobenzenesulphonyl morpholide	Neg	Neg	Neg	Neg	Neg	Neg
ESS 28	*p*-Toluenesulphonyl morpholide	Neg	Neg	Neg	Neg	Neg	Neg
Nitro compounds							
UT 10	2,3-Dinitro-2,3-dimethylbutane	Neg	Neg	Neg	Neg	Neg	Neg
ESS 62	3,5-Dinitro-*o*-cresol	5.5	15	6	11	140	270
ESS 46	β-Nitrostyrene	90	220	90	180	Neg	Neg
ESS 20	*o*-Nitrobiphenyl	250	Neg	Neg	Neg	Neg	Neg
ESS 21	*p*-Nitrobiphenyl	360	Neg	Neg	Neg	Neg	Neg
UT 13	2,4-Dinitro-6-cyclohexylphenol	16	75	6	13	300	Neg

TABLE II—*Concluded*

APPROXIMATE MEDIAN LETHAL DEPOSITS (LD_{50}) AND LD_{90} FIGURES OF 92 COMPOUNDS FOR NYMPHS OF *Blatella* AND *Oncopeltis*, AND ADULTS OF *Tribolium*—Continued

Expressed in gamma per sq. cm.

No.	Name of compound	*Blatella*		*Oncopeltis*		*Tribolium*	
		LD_{50}	LD_{90}	LD_{50}	LD_{90}	LD_{50}	LD_{90}
Miscellaneous nitrogenous compounds							
UT 9	Dibutylnitramine	180	Neg	Neg	Neg	Neg	Neg
UT 5	Dicyanodiethylnitramine	Neg	Neg	Neg	Neg	Neg	Neg
UT 11	Di-*n*-butylcyanamide	155	300	Neg	Neg	Neg	Neg
UT 12	*p,p'*-Dichlorobenzophenone oxime	Neg	Neg	Neg	Neg	Neg	Neg
ESS 22	*o*-Aminobiphenyl	115	235	Neg	Neg	Neg	Neg
ESS 91	Thiourea	370	Neg	Neg	Neg	Neg	Neg
Compounds containing C, H, and O only							
ESS 58	Cyclohexylacetic acid	280	Neg	380	Neg	Neg	Neg
ESS 59	Cyclohexylpropionic acid	180	Neg	Neg	Neg	Neg	Neg
ESS 60	Cyclohexylbutyric acid	160	Neg	Neg	Neg	Neg	Neg
ESS 61	Cyclohexylcaproic acid	Neg	Neg	Neg	Neg	Neg	Neg
ESS 23	*o*-Hydroxybiphenyl	60	155	Neg	Neg	Neg	Neg
ESS 3	Coumarin	260	Neg	Neg	Neg	Neg	Neg
Thiocyanates							
ESS 41	Benzyl thiocyanate	35	70	80	170	Neg	Neg
ESS 105	*o*-Chlorobenzyl thiocyanate	45	90	55	130	Neg	Neg
ESS 106	*p*-Chlorobenzyl thiocyanate	30	80	33	55	Neg	Neg
ESS 136	2,4-Dichlorobenzyl thiocyanate	380	Neg	50	90	Neg	Neg
ESS 138	3,4-Dichlorobenzyl thiocyanate	Neg	Neg	70	105	Neg	Neg
ESS 102	α-Thiocyano-1-methylnaphthalene	Neg	Neg	Neg	Neg	Neg	Neg
UT 14	Isoborneyl thiocyanoacetate (Fraction 2)	130	Neg	90	120	Neg	Neg
UT 15	Isoborneyl thiocyanoacetate (Fraction 5)	Neg	Neg	—	—	Neg	Neg
Compounds containing other elements							
UT 17	Phenylboric acid	Neg	Neg	—	—	Neg	Neg
UT 18	*o*-Nitrophenylboric acid	Neg	Neg	—	—	Neg	Neg
ESS 68	Tri-*o*-cresyl phosphate	Neg	Neg	—	—	Neg	Neg

Discussion of Results

The resistance of adults of *Tribolium confusum* to contact sprays of the organic compounds tested is remarkable, since the great majority of them caused no mortality whatsoever. Only five compounds, namely DDT, gammexane, chlordane, DNOC, and DNOCHP, were powerful enough to induce a significant degree of mortality. All of these are proven insecticides, and moreover are toxic to *Tribolium* at quite low dosages.

Both gammexane and chlordane showed a consistently high level of toxicity to the three species tested. The toxicity of gammexane to *Blatella germanica* has already been reported in general terms by Slade (8). Good results with gammexane applied as a contact insecticide have recently been communicated by Bottger and Levin (2) for agricultural insects. The superiority of chlordane as a contact poison for the cockroach *Periplaneta americana* has been reported by Kearns, Ingle, and Metcalf (6), who found this material to be superior to DDT against many species of insects.

Although DDT was found to be the most toxic of the compounds tested against *Tribolium* adults, it proved to be one of the less toxic materials for *Blatella* nymphs. Moreover it exhibited no toxicity whatever* to *Oncopeltis* nymphs. The nontoxicity of DDT to certain insect species has been noted by many workers. None of the analogues of DDT tested showed any appreciable toxicity to either *Blatella* or *Tribolium*, although methoxy-DDT was moderately toxic, and ethoxy-DDT slightly toxic, to *Oncopeltis*.

These results show that DNOC (3,5-dinitro-*o*-cresol) is an effective contact insecticide for the German cockroach, as it is to grasshoppers and locusts, related orthopterans. Moreover it shows considerable contact toxicity to nymphs of *Oncopeltis*, representing the order Hemiptera. It is followed in toxicity by 2,4-dinitro-6-cyclohexylphenol, a nitro compound cited as being generally superior to DNOC by Kagy (5). β-Nitrostyrene was a third nitro compound to show high contact toxicity.

The most promising of the compounds newly synthesized for this project were benzyl thiocyanate and its *o*- and *p*-chloro derivatives. These three thiocyanates showed greater contact toxicity than Lethane 60 or Lethane 384, which were tested for comparison. They were also superior to a sample of isoborneyl thiocyanoacetate obtained from the insecticidal material Thanite. Benzyl thiocyanate has been found by Hartzell and Wilcoxon (4) to be an effective contact insecticide for *Aphis rumicis*, although it formed poor emulsions. None of the thiocyanates showed toxicity to *Tribolium* adults.

A considerable number of the cyanides and nitriles tested showed moderate contact toxicity to *Blatella* nymphs. Benzyl cyanide (phenylacetonitrile), phenylpropionitrile, styryl cyanide, the *o*- and *p*-chlorobenzyl cyanides and 3,4-dichlorobenzyl cyanide, all showed median lethal concentrations of between 120 and 35 gamma per cm.^{2} However, none had any effect on *Tribolium* adults, and only *p*-chlorobenzyl cyanide showed slight toxicity to *Oncopeltis* nymphs.

It is of interest to note that the α-chlorotoluene analogues, namely the chlorobenzyl and dichlorobenzyl chlorides as well as benzotrichloride, showed a negligible degree of contact toxicity to any of the three species tested. Moreover the group of halogenated aliphatic compounds, many of which have shown a general and fumigant toxicity of a high order, exhibited little or no effect as contact sprays.

*_However, at the end of the four-day observation period, the insects did exhibit varying degrees of DDT jitters._

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